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Detection and Activity of Lactacin B, a Bacteriocin Produced by *Lactobacillus acidophilus*†

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A total of 52 strains of *Lactobacillus acidophilus* were examined for production of bacteriocins. A majority (63%) demonstrated inhibitory activity against all members of a four-species grouping of *Lactobacillus leichmannii, Lactobacillus bulgaricus, Lactobacillus helveticus*, and *Lactobacillus lactis*. Four *L. acidophilus* strains with this activity also inhibited *Streptococcus faecalis* and *Lactobacillus fermentum*, suggesting a second system of antagonism. Under conditions eliminating the effects of organic acids and hydrogen peroxide, no inhibition of other gram-positive or -negative genera was demonstrated by *L. acidophilus*. The agent produced by *L. acidophilus* N2 and responsible for inhibition of *L. leichmannii, L. bulgaricus, L. helveticus*, and *L. lactis* was investigated. Ultrafiltration studies indicated a molecular weight of approximately 100,000 for the crude inhibitor. The agent was sensitive to proteolytic enzymes and retained full activity after 60 min at 100°C (pH 5). Activity against sensitive cells was bactericidal but not bacteriolytic. These characteristics identified the inhibitory agent as a bacteriocin, designated lactacin B. Examination of strains of *L. acidophilus* within the six homology groupings of Johnson et al. (Int. J. Syst. Bacteriol. **30**:53–68, 1980) demonstrated that production of the bacteriocin lactacin B could not be used in classification of neotype *L. acidophilus* strains. However, the usefulness of employing sensitivity to lactacin B in classification of dairy lactobacilli is suggested.

Since Metchnikoff (19) proposed a role for lactobacilli in suppressing undesirable intestinal microflora, numerous researchers have investigated the antimicrobial activities of *Lactobacillus acidophilus*. Broad-spectrum inhibition has been clearly demonstrated for organic acids and hydrogen peroxide produced by *L. acidophilus* (5, 28). In addition, a number of reports suggest that antimicrobial proteins, or bacteriocins, either mediate or facilitate antagonism by *L. acidophilus* (5, 8, 9, 20, 23, 31). Vincent et al. (31) first described a bacteriocin-type inhibitor produced in aged liver veal agar cultures of *L. acidophilus*. Crude "lactocidin" was nonvolatile, non-dialyzable, insensitive to catalase, and active at neutral pH. It displayed inhibitory activity against numerous genera, including *Proteus, Salmonella, Escherichia, Staphylococcus, Bacillus, Streptococcus*, and *Lactobacillus*. Because of the broad activity spectrum of lactocidin, Vincent et al. concluded that *L. acidophilus* occupies an important position in controlling undesirable microflora in the intestinal tracts of humans and animals. No further studies on lactocidin, or similar broad-spectrum bacteriocins produced by *L. acidophilus*, have been reported.

Bacteriocins, by definition, possess little potential for broad-spectrum inhibition. Criteria for their identification include an activity restricted to closely related species, a bactericidal mode of action, and a proteinaceous nature (26). Although a broader range of activity has been detected for a limited number of bacteriocins produced by gram-positive bacteria, studies that definitely identify bacteriocins produced by lactobacilli report inhibitory activities restricted to the *Lactobacilliaceae* (2–4, 29, 30).

Previous studies of antagonism by *L. acidophilus* do not specifically address, identify, or confirm the involvement of bacteriocins (10, 13). In this study, 52 strains of *L. acidophilus* were examined for inhibition of gram-positive and -negative bacteria under conditions eliminating antagonism by organic acids or hydrogen peroxide. Sensitivity to *L. acidophilus* was identified only among members of the *Lactobacilliaceae* and group D streptococci. Of several inhibitory interactions detected, that of *L. acidophilus* N2 toward *Lactobacillus leichmannii, Lactobacillus bulgaricus, Lactobacillus lactis*, and *Lacto-*

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bacillus helveticus was examined. The inhibitory agent, designated lactacin B, was identified as a bacteriocin.

MATERIALS AND METHODS

Bacterial cultures. Strains of *L. acidophilus* (Table 1), indicator lactobacilli (Table 2), and other indicator strains (Table 3) were obtained from the stock culture collection maintained by the Department of Food Science at North Carolina State University or other sources as indicated. Identity of lactobacilli was verified via Gram stain, catalase test, growth at 15 and 45°C, and carbohydrate fermentation patterns (6, 21). Esculin hydrolysis and production of ammonia from arginine were examined by the procedure of Davis (1).

All cultures were maintained as frozen stocks at −76°C (14). Before experimental use, cultures were propagated twice in broth media for 14 to 16 h. Lactobacilli were propagated in MRS broth (Difco Laboratories, Detroit, Mich.) at 37°C. Group N streptococci were propagated in Elliker broth (Difco Laboratories) at 30°C. All other indicator cultures were propagated in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C. Transfer inoculum was 1% for all cultures.

Agar media were prepared by adding 1.5% granulated agar (BBL Microbiology Systems) to the broth media listed above. Soft or overlay agars were prepared with 0.75% agar.

Bacteriocin detection. *L. acidophilus* cultures were screened for bacteriocin production by both direct (26) and deferred (12) methods. For the direct method, colonies of *L. acidophilus* were stabbed into MRS agar plates previously overlaid with an MRS soft agar (0.75%) lawn of the indicator culture. Indicator lawns were prepared by adding 0.25 ml of a 10^{-1} dilution from an overnight culture to 10 ml of MRS soft agar. The contents of the tube were gently mixed and poured over the surfaces of prepoured MRS agar plates (150 by 15 mm). After stabbing with multiple *L. acidophilus* strains, plates were incubated for 24 h at 37°C in an anaerobic Gas-Pak system (H_2 + CO_2 generator; BBL Microbiology Systems). After incubation, the indicator lawns were examined for zones of inhibition surrounding each producer stab. In the deferred method, *L. acidophilus* broth cultures were streaked across the surface of prepoured MRS agar plates. After incubation for 24 h at 37°C under anaerobic conditions, the agar was inverted into the cover of the petri dish. The entire uninoculated surface was then seeded with the indicator culture. These plates were incubated at 37°C under anaerobic conditions and examined for inhibition of indicator growth.

Preparation of culture extracts. Eighteen-hour MRS broth cultures of *L. acidophilus* were swabbed across

TABLE 1. Strains of *L. acidophilus* examined for antagonism

Strain no.	Origin[a]	Reference(s)
5, 1375, 1693, 1697	NCDO	
4, 104, 860	NCDO	22
MS01, MS02, MS03, MS04, MS05, MS06, MS07, MS08, MS09, MS10, MS11, N2	NCSU	
PA3, PA19, P47	NCSU	7
RL8K, 1	NCSU	14
6032 (neotype),[b] 11084, 11085, 11760-B, 12596, 1784, 11083, 0607-1B, 7635, 11761, 0818, 1756, 1830, P10B-17, 1793, 6033, 11092, 12601, 11759, 7960, 11088, 11694, 11696, 12600	VPI	11
1754, 1294, 2164-A, 11089	VPI	11, 22

[a] Abbreviations: NCDO, National Collection of Dairy Organisms, National Institute for Dairying, Reading, England; NCSU, Department of Food Science Culture Collection, North Carolina State University, Raleigh, N.C.; VPI, J. L. Johnson, Virginia Polytechnic Institute, Blacksburg, Va.

[b] Also listed as NCDO 1748 and ATCC 4356 (ATCC, American Type Culture Collection, Rockville, Md.).

TABLE 2. Indicator lactobacilli

Strain no.	Origin[a]
L. acidophilus 6032 (neotype)	VPI
L. bulgaricus 1489 (neotype)	NCDO
L. casei 7469	ATCC
L. fermentum 1750 (neotype)	NCDO
L. helveticus 87	NCDO
L. lactis 970	NCDO
L. leichmannii 4797 (neotype)	ATCC
L. plantarum 1752 (neotype)	NCDO
L. viridescens 12706 (neotype)	ATCC

[a] Abbreviations as in Table 1, footnotes *a* and *b*.

TABLE 3. Non-lactobacilli indicators

Organism	Origin[a]
Bacillus cereus	Collins[b]
Clostridium perfringens 3624	ATCC
Citrobacter freundii 8090	ATCC
Enterobacter aerogenes 13048	ATCC
Enterobacter cloacae 23355	ATCC
Escherichia coli 25922	ATCC
Escherichia coli O:24 B:17, enteropathogenic	NCSU
Klebsiella pneumoniae 13883	ATCC
Proteus vulgaris 13315	ATCC
Pseudomonas aeruginosa 27853	ATCC
Salmonella typhimurium 14028	ATCC
Serratia marcescens 8100	ATCC
Shigella flexneri 14028	ATCC
Shigella sonnei 25931	ATCC
Staphylococcus aureus 25923	ATCC
Staphylococcus epidermidis 12228	ATCC
Streptococcus cremoris 799	NCDO
Streptococcus faecalis 19433	ATCC
Streptococcus lactis C2	McKay[c]
Streptococcus pyogenes 19615	ATCC

[a] Abbreviations as in Table 1, footnotes *a* and *b*.

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[c] L. L. McKay, Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minn.

the entire surface of MRS agar plates (150 by 15 mm). After anaerobic incubation for 48 h at 37°C, the agar was removed from the petri dish, weighed, and added to an equal weight of sterile phosphate buffer (0.3 mM NaH_2PO_4-Na_2HPO_4, pH 7.0). The buffer-agar mixture was crushed by hand in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) and then allowed to equilibrate for 24 h at 4°C. After prefiltration through Whatman no. 1 filter paper (Whatman Ltd., Kent, England), remaining agar and bacterial cells were removed by centrifugation at 10,300 × *g* for 10 min at 4°C. The supernatant was adjusted to pH 6.0, sterilized by passage through a 0.45-μm Acrodisc filter (Gelman Sciences, Inc., Ann Arbor, Mich.), and stored at −76°C. Where indicated, supernatants of the culture extract were exhaustively dialyzed against 0.3 mM phosphate buffer (Na_2HPO_4-NaH_2PO_4, pH 7.0) in Spectrapor no. 2 membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, Calif.) before filter sterilization and storage.

Bacteriocin detection in culture extracts was performed by the method of Tagg and McGiven (27). Wells were cut in MRS agar plates with a sterile 7-mm cork borer and sealed at the bottom by the addition of 1 to 2 drops of agar. About 2 to 3 drops of the culture extract were placed in the sealed wells. After diffusion of the extract into the agar (6 h at 25°C), the agar was inverted, and the exposed surface was overlaid with an indicator lawn. Indicator lawns were prepared as described above, except that MRS, Elliker, and brain heart infusion overlay soft agars were used for the lactobacilli, group N streptococci, and all other indicator bacteria, respectively.

For some experiments, culture extracts were supplemented with 68 U of filter-sterilized catalase (3,390 U/mg; Sigma Chemical Co., Saint Louis, Mo.) per ml. Activity was determined as above except that sterile catalase (68 U/ml) was added to both agar media and soft overlay agars before testing.

Activity assay. Bacteriocin activity was assayed by an adaptation of the critical dilution method used for the assay of bacteriocins (18). MRS agar plates were overlaid with MRS agar (0.75%) lawns containing approximately 10^5 CFU of a log-phase culture of *L. leichmannii* 4797 per ml. Portions (20 μl) of serial twofold dilutions of culture extracts were spotted onto these lawns. After 14 to 16 h at 37°C under flowing CO_2 (0.4 liters/h), plates were examined for inhibition of the bacterial lawn. The extract titer was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn and was expressed in activity units (AU) per milliliter.

Protease sensitivity. Culture extracts containing lactacin activities were treated with 500 μg of *Streptomyces griseus* protease (Sigma Chemical Co.) per ml. Samples with and without protease were filter sterilized and held at 37°C for 1 h. Activity remaining in both samples after protease digestion was detected by agar-well diffusion against a sensitive indicator lawn.

Bactericidal action. Culture extracts containing lactacin B activity were dialyzed against 0.3 mM phosphate buffer (pH 7.0), concentrated to one-tenth volume with polyethylene glycol (Carbowax PEG 20,000; Fisher Scientific Co., Raleigh, N.C.), and sterilized by passage through a 0.45-μm filter. Samples of the concentrated extracts (256 AU/ml) were diluted with sterile 0.3 mM phosphate buffer (pH 7.0) to 0, 4, 12, and 50 AU/ml. A 15-ml sample of each preparation was added to a separate sterile cuvette and prewarmed to 37°C. Cells from a log-phase culture of *L. leichmannii* 4797 (5 h, 37°C) were washed in phosphate buffer and added to each cuvette at an initial population of 9.6×10^7 CFU/ml. At intervals of 0, 30, 60, and 90 min, absorbance at 650 nm was determined, and 2-ml samples were removed for viable counts. Surviving cells were enumerated on MRS agar as described previously (14).

Mutant selection and plasmid analysis. For selection of mutants lacking lactacin B activity, *L. acidophilus* PA3 was propagated for 18 h at 37°C in MRS broth containing various amounts of ethidium bromide (0 to 30 μg/ml; Sigma Chemical Co.). These cultures were serially diluted and surface plated on MRS agar to obtain uncrowded colonies (30 to 50 per plate). The plates were immediately overlaid with 15 ml of fresh MRS agar. After 24 h at 37°C under anaerobic conditions, the plates were overlaid with a cell lawn of *L. leichmannii* 4797 and incubated as described above. After development of the cell lawn, single colonies were examined for surrounding zones of inhibition. Plasmid analysis was conducted as described previously (15).

RESULTS

Inhibitory spectra. Extracts from 53 different *L. acidophilus* agar cultures were examined for inhibition of the indicator strains listed in Tables 2 and 3. With the single exception of *Streptococcus faecalis* 19433, sensitive indicator strains were limited exclusively to members of the *Lactobacilliaceae* (Table 4). A total of 33 strains displayed a common inhibitory activity that affected four *Lactobacillus* indicators, *L. leichmannii* 4797, *L. bulgaricus* 1489, *L. helveticus* 87, and *L. lactis* 970 (Table 4). Fourteen of these strains showed expanded inhibitory activity against the neotype strain, *L. acidophilus* 6032. *L. acidophilus* strains 11759, 11088, 11089, and MS01 demonstrated a third activity effective against *L. fermentum* 1750 and *S. faecalis* 19433. During these studies, *L. acidophilus* strains were further examined for sensitivity to their own products in culture extracts and for autoinhibition by direct antagonism testing. Producer strains of *L. acidophilus* were not autoinhibitory. The high proportion of *L. acidophilus* strains (81%) that inhibited the indicator group of *L. leichmannii* 4797, *L. bulgaricus* 1489, *L. helveticus* 87, and *L. lactis* 970 suggested that production of the antagonistic agent was characteristic of *L. acidophilus* species. Although the results in Table 4 implicated a number of potential bacteriocin systems, further studies in this investigation were confined to the antagonistic reaction occurring between *L. acidophilus* and the four *Lactobacillus* indicators.

Characteristics of lactacin B. To identify the agent responsible for antagonism of the four *Lactobacillus* indicators, *L. acidophilus* N2 was

TABLE 4. Inhibition of indicator species by *L. acidophilus*

L. acidophilus[a]	Inhibition of indicator strain:[b]						
	L. leichmannii 4797	*L. bulgaricus* 1489	*L. lactis* 970	*L. helveticus* 87	*L. acidophilus* 6032	*L. fermentum* 1750	*S. faecalis* 19433
MS01, 11088, 11089, 11759	Yes	Yes	Yes	Yes	Yes	Yes	Yes
104, 1697, MS03, MS04, MS06, MS07, MS08, PA3, P47, RL8K, 1, 11760-B, P10B-17, 7960	Yes	Yes	Yes	Yes	Yes		
4, 5, 1375, MS05, MS09, MS11, N2, PA19, 6032, 11084, 11085, 11083, 7635, 6033, 12596	Yes	Yes	Yes	Yes			
1294	Yes	Yes	Yes				
860, 1693		Yes	Yes	Yes			
1784	Yes	Yes					
MS10, 1754		Yes	Yes				
0818, 0607-1B				Yes			
12600	Yes			Yes			

[a] Culture extracts were prepared from anaerobic agar cultures, neutralized to pH 6.0, and tested for activity by the method of Tagg and McGiven (27).

[b] All positive reactions were confirmed by further direct or deferred (or both) antagonism testing.

selected as a model system for further study. *L. acidophilus* N2 consistently inhibited *L. leichmannii* 4797, *L. bulgaricus* 1489, *L. helveticus* 87, and *L. lactis* 970 during direct or deferred antagonism on agar and in agar-well diffusion of culture extracts (Fig. 1). Activity was detected only in agar cultures. Cell-free supernatants from MRS broth cultures of *L. acidophilus* were not inhibitory to the sensitive indicator species.

Activity of the inhibitor produced by *L. acidophilus* N2, designated lactacin B, was eliminated by treatment with 500 μg of protease per ml for 1 h at 37°C. Preparations without protease retained full activity. In addition, culture extracts (pH 5.0) containing lactacin B activity were unaffected by catalase or heating at 100°C for 1 h. These results demonstrated that the inhibitor produced by *L. acidophilus* N2 was a heat-stable protein.

Lactacin B activity in culture extracts was retained during exhaustive dialysis against 0.3 mM phosphate buffer (pH 7.0) in membrane tubing with molecular exclusion limits of 12,000 to 14,000 daltons. During ultrafiltration of lactacin B in 0.3 mM phosphate buffer, pH 7.0, a 75% loss of activity was observed. Of the remaining activity, 92.3% was retained by an XM300 Diaflo membrane (Amicon, Danvers, Mass.) and 7.7% by an XM100A Diaflo membrane. Molecular exclusion limits for the XM300 and XM100A membranes are 3×10^5 and 1×10^5, respectively. The retention of lactacin B by these membranes suggests that its size exceeds 10^5. However, activity losses could not be eliminated, suggesting that the conditions encountered during ultrafiltration could have resulted in protein aggregation or irreversible adsorption to the Diaflo membranes. Alternatively, the compound may be unstable under the buffering conditions employed.

Bactericidal action of lactacin B. To test for a bactericidal or bacteriolytic mode of action, we investigated the effects of lactacin B on the viability and lysis of *L. leichmannii* 4797. The addition of increasing concentrations of lactacin B to *L. leichmannii* 4797 cells resulted in proportional increases in cell death (Fig. 2). Within 30 min, viability of the cell population was reduced by 87% at 4 AU/ml; at higher concentrations, reductions in CFU per milliliter exceeded 99.9%. Optical densities of the test cell suspensions were stable throughout the experiment, and cell lysis was not detected during microscopic observations. Lactacin B acted rapidly on *L. leichmannii* 4797 with a bactericidal mode of action.

Adsorption studies. The lethal action of bacteriocins occurs as a two-step process initiated by adsorption of the protein to sensitive cells (26). Lactacin B was examined for adsorption to both sensitive and insensitive *Lactobacillus* species. Table 5 shows that producer strains *L. acidophilus* 6032 and N2 did not adsorb lactacin B, whereas all four sensitive strains adsorbed 75 to 100% of the activity. Possibly, resistance of producer cells to lactacin B may reside in the absence or masking of cell receptors. However, *L. casei* 7469, *L. fermentum* 1750, *Lactobacillus plantarum* 1752, and *Lactobacillus viridescens* 12706, which are insensitive to lactacin B, also adsorbed the bacteriocin at levels comparable to sensitive indicator species. These results suggested that the specificity of lactacin B action was not entirely dependent on the presence of particular receptors on sensitive cells.

Plasmid involvement. Plasmid DNA elements

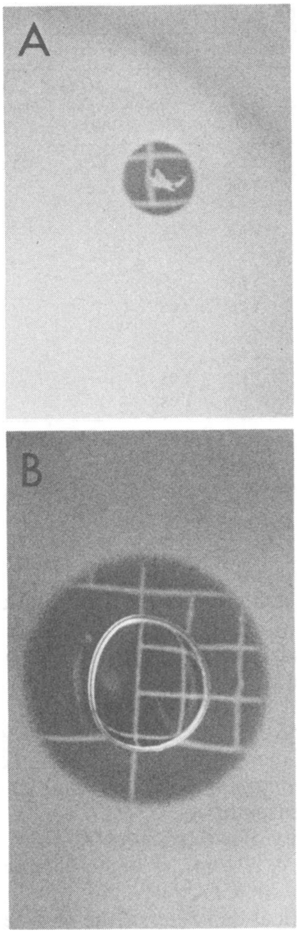

FIG. 1. (A) Inhibition of *L. leichmannii* 4797 by *L. acidophilus* N2 stab culture via direct method for demonstrating antagonism. (B) Culture extracts of *L. acidophilus* N2 tested by the agar-well diffusion method of Tagg and McGiven (27).

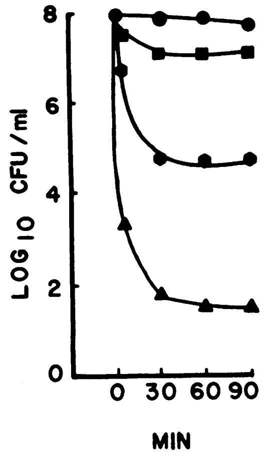

FIG. 2. Bactericidal activity of lactacin B against *L. leichmannii* 4797. Crude lactacin B (pH 7.0) was added at concentrations of 0 (●), 4 (■), 12 (●), and 50 (▲) AU/ml to sterile 0.3 mM phosphate buffer. Cells from 5-h MRS broth cultures were washed in buffer and added to a final concentration of 9.6×10^7 CFU/ml. The optical density at 650 nm was determined spectrophotometrically, and survivors (CFU per milliliter) were determined by plating on MRS agar.

commonly participate as genetic determinants for bacteriocin production (16, 26). However, attempts to demonstrate the presence of plasmid DNA in *L. acidophilus* N2 were unsuccessful (data not shown). In lieu of a potential plasmid system in *L. acidophilus* N2, *L. acidophilus* PA3 was selected for curing studies of lactacin B activity. *L. acidophilus* PA3 has been shown previously to carry two plasmids (13.7 and 6.3 megadaltons; see reference 15) and demonstrates lactacin B activity against the four sensitive *Lactobacillus* indicators (Table 4). Mutants deficient in lactacin B activity could not be isolated spontaneously (2,049 colonies examined) or after growth in the presence of ethidium bromide (2,075 colonies examined). The absence of plasmid DNA in *L. acidophilus* N2 and the stability of the activity in a plasmid-carrying strain, *L. acidophilus* PA3, implicated a chromosomal determinant for lactacin B.

Taxonomy. The antagonism of a majority of *L. acidophilus* strains toward a four-species group of lactobacilli suggested that production of and sensitivity to lactacin B may be significant taxonomic characteristics. Bacteriocins, via production or sensitivity, have been routinely used as taxonomic markers for bacterial species (26). To determine if sensitivity to lactacin B was common to strains of *L. leichmannii*, seven strains (including *L. leichmannii* 4797) were tested for inhibition by *L. acidophilus* N2 by both direct and deferred methods. Culture sensitivity was also confirmed with agar-well diffusion assays. Under all conditions, the seven strains of *L. leichmannii* were sensitive to *L. acidophilus* N2 and its culture extract containing lactacin B. Furthermore, all strains of *L. bulgaricus* (9), *L. helveticus* (2), and *L. lactis* (7) tested were sensitive to lactacin B. Although a limited number of sensitive strains were tested, it appeared that lactacin B from *L. acidophilus* N2 was active against all strains within sensitive-species groups.

Production of a lactacin B-type activity by 33 strains of *L. acidophilus* (Table 4) suggested potential taxonomic significance in classifying *L. acidophilus* species. Twenty-eight *L. acidophilus* strains, classified previously by Johnson et al. (11) into six DNA homology groups, were examined for activity against the lactacin B

TABLE 5. Adsorption of lactacin B by selected lactobacilli

Test culture[a]	Lactacin B (AU/ml)	
	Initial	Remaining
L. acidophilus N2	4	4
L. acidophilus 6032	4	4
L. bulgaricus 1489	4	1
L. helveticus 87	4	0
L. lactis 970	4	1
L. leichmannii 4797	4	0
L. casei 7469	4	0
L. fermentum 1750	4	1
L. plantarum 1752	4	1
L. viridescens 12706	4	0
None[b]	4	4
L. acidophilus N2	0	0
L. acidophilus 6032	0	0

[a] Cells from overnight MRS broth cultures were harvested by centrifugation, washed twice in 0.3 mM sodium acetate (pH 5.0), and resuspended in fresh acetate buffer containing 4 AU of lactacin B per ml. Cell concentrations ranged between 10^8 and 10^9 CFU/ml. After 1 h of incubation on ice, the bacterial cells were removed by filtration, and the remaining lactacin B activity was determined in the cell-free filtrate.

[b] Control experiments included incubation of the lactacin B-acetate buffer in the absence of cells and incubation of producer cells in acetate buffer only.

indicators (Table 6). All strains in homology group A1 exhibited activity against all four lactacin B indicators. Strains within homology groups A2, A3, A4, B1, and B2 exhibited no consistent pattern of inhibition. Some strains showed no activity, whereas others showed activity against one or more of the indicators. The absence of any consistent pattern of inhibition indicated that the production of lactacin B or related activities in *L. acidophilus* could not be employed as a taxonomic marker for *L. acidophilus* strains.

DISCUSSION

Results from this investigation clearly demonstrated that antagonism by *L. acidophilus* was restricted to selected members of the *Lactobacilliaceae* under conditions eliminating the effects of organic acids and hydrogen peroxide. With the exception of *S. faecalis* 19433, all unrelated gram-positive and -negative bacteria tested were insensitive to *L. acidophilus*. Although two *L. acidophilus* strains in this study have been reported to produce lactocidin (31), our findings confirmed only an activity against other lactobacilli. In contrast to previous reports, we observed no broad-spectrum inhibitory activity for either strain when hydrogen peroxide and organic acids were eliminated. Therefore, although *L. acidophilus* has been reputed to exhibit natural antibiotic activities (24), data from Spillman et al. (25) and this study do not substantiate reports of broad-spectrum antibiotic production by *L. acidophilus*.

Since the report of Vincent et al. (31), a number of studies on *L. acidophilus* have attempted to define antagonistic agents other than lactic acid and hydrogen peroxide (8, 9, 20). The responsible agents have not been identified (20) or have not met the basic criteria for identification of bacteriocins (10, 13). In the present

TABLE 6. Lactacin B activity of 28 strains of *L. acidophilus* characterized within six DNA-DNA homology groups

Homology group[a]	*L. acidophilus* strain designation	Inhibition of indicator strains:[b]			
		L. leichmannii 4797	*L. bulgaricus* 1489	*L. lactis* 970	*L. helveticus* 87
A1	6032	+	+	+	+
	11084	+	+	+	+
	11085	+	+	+	+
	11760-B	+	+	+	+
	12596	+	+	+	+
A2	1784	+	+	−	−
	11083	+	+	+	+
	0607-1B	−	−	−	+
	7635	+	+	+	+
	11761	−	−	−	−
A3	1754	−	+	+	−
	0818	−	−	−	−
	P10B-17	+	+	+	+
	1756	−	−	−	−
	1830	−	−	−	−
A4	1294	+	+	+	−
	1793	−	−	−	−
	2164A	−	−	−	−
B1	6033	+	+	+	+
	11092	−	−	−	−
	11089	+	+	+	+
	12601	−	−	−	−
	11759	+	+	+	+
B2	7960	+	+	+	+
	11088	+	+	+	+
	11694	−	−	−	−
	11696	−	−	−	−
	12600	+	−	−	+

[a] Homology groupings of Johnson et al. (11).

[b] Culture extracts of *L. acidophilus* strains were tested for bacteriocinic antagonism by the method of Tagg and McGiven (27).

[c] +, Denotes inhibition; −, denotes no inhibition.

study, we identified *L. acidophilus* strains that demonstrate marked antagonism against six sensitive indicators: *L. leichmannii, L. bulgaricus, L. helveticus, L. lactis, L. fermentum*, and *S. faecalis*. The inhibitors produced by *L. acidophilus* were produced and active under anaerobic conditions, unaffected by neutral pH, and retained during dialysis. Furthermore, the agents were insensitive to catalase, diffused readily through agar, and did not form plaques during dilution to extinction. With the elimination of organic acids, hydrogen peroxide, or bacteriophage, the inhibitory spectra observed for the 52 *L. acidophilus* strains examined strongly suggested the involvement of at least three distinct bacteriocin-type activities. However, confirmation of bacteriocin activity was limited, in the present study, to characterization of the inhibitor produced by *L. acidophilus* N2, lactacin B.

Similar to bacteriocins that have been characterized previously (16, 26), lactacin B was a large, heat-stable protein produced exclusively in agar cultures. Activity against *L. leichmannii* 4797 was clearly bactericidal, and death occurred within 30 min of exposure to lactacin B. Population reductions were finite and proportional to the concentration of lactacin B present. According to a single-hit mechanism for bacteriocin action (26), bacteriocins sequentially adsorb to, penetrate, and kill sensitive cells. This process is irreversible and nonpropagating, thereby resulting in finite levels of cell death proportional to the bacteriocin concentration. Lactacin B adsorbed to sensitive cells and acted in a single-hit fashion characteristic of bacteriocinic proteins.

Bacteriocin adsorption is a critical step in initiation of lethal interactions between these proteins and sensitive cells (26). Unlike a majority of bacteriocins which demonstrate specific adsorption to sensitive cells, lactacin B adsorbed to both sensitive and insensitive *Lactobacillus* species. Adsorption of lactacin B demonstrated a functional property characteristic of bacteriocins. However, the lack of adsorption specificity indicates that cell sensitivity or resistance is not solely determined by the presence or absence of specific cell receptors for lactacin B. Nonlethal binding has been reported for a number of gram-positive bacteriocins, including lactocin 27 produced by *L. helveticus* (26, 29, 30). Alternatively, lactacin B did not adsorb to producer cells. Therefore, autoimmunity of producer cells to lactacin B could result from either the absence or masking of cell receptors for adsorption. In direct contrast to the nonspecific adsorption responses of lactacin B to sensitive and resistant lactobacilli, adsorptive exclusion by producer cells demonstrated some specificity for lactacin B. This apparent specificity raises two possibilities. Either resistant lactobacilli may offer competitive, nontarget receptors that adsorb lactacin B, or, after adsorption to a target receptor on resistant cells, the bacteriocin is inactivated or incapable of additional lethal activity. Further studies are in progress to determine the mechanisms for lactacin B specificity, resistance, and its action on specific molecular targets within sensitive cells.

The spectrum of activity for lactacin B was not directed toward diverse *Lactobacillus* species, but was restricted to phylogenetically related species. DNA homology studies have demonstrated that substantial genetic relatedness exists between *L. leichmannii, L. bulgaricus, Lactobacillus delbrueckii, L. lactis, L. helveticus*, and *L. acidophilus* (17). Those species insensitive to lactacin B, *L. plantarum, L. casei, L. viridescens*, and *L. fermentum*, are considered phylogenetically more distant from *L. acidophilus* than are the species sensitive to lactacin B (17). Therefore, lactacin B demonstrated activity against those species that are most closely related to *L. acidophilus*. Action within or against closely related species provided additional evidence warranting classification of lactacin B as a bacteriocin. Additionally, bacteriocins, via production or sensitivity, have been employed as taxonomic markers for identification of bacterial species (26). Production of a lactacin B activity was not taxonomically significant due to its variability throughout *L. acidophilus* homology groups. However, species sensitivity to lactacin B was consistently observed for strains of *L. leichmannii, L. bulgaricus, L. lactis*, and *L. helveticus*. Therefore, sensitivity to lactacin B may be a useful characteristic in the taxonomic identification of these *Lactobacillus* species.

Detection and description of lactacin B provides the first evidence for bacteriocin production by *L. acidophilus* since the report of Vincent et al. (31). Although broad-spectrum activities similar to those described by Vincent et al. were not detected in this study, a number of specific antagonistic interactions were observed that potentially could involve bacteriocins other than lactacin B. Inhibition of *L. fermentum* and *S. faecalis* by four *L. acidophilus* strains producing a lactacin B activity also suggests production of an additional bacteriocin or bacteriocins. Similarly, inhibition of *L. acidophilus* 6032 by select *L. acidophilus* producers may also involve a bacteriocin. Unquestionably, definition of multiple bacteriocins in *L. acidophilus* will require additional studies emphasizing mutant analysis and molecular characterization of the inhibitory compounds involved in specific producer-indicator interactions.

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